Photosynthetic characteristics of *Zea mays* seedlings grown under controlled environmental conditions

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Studies were carried out on the effect of irradiance, illumination time and limited water and nutrient application on the activities of the carboxylating enzymes in relation to leaf position in *Zea mays* L. seedlings. The ratio phosphoenolpyruvate (PEP) carboxylase:ribulose-1,5-biphosphate (RuBP) carboxylase increased in the first-formed leaves with increased irradiance, and varied markedly in these leaves throughout the photoperiod. Conditions of water and nutrient deprivation reduced the activity of PEP carboxylase to a greater extent than RuBP carboxylase, in all the leaves of 3-week old plants.

The initial photosynthetic products and $\delta^{13}C$ values were investigated in 3-week old seedlings. $^{14}CO_2$ was fixed predominantly into C_4 acids in all leaves, and these leaves also had $\delta^{13}C$ values of $-11{,}5‰$.

The results indicate that growth conditions influence the photosynthetic characteristics of individual leaves, and that the C_4 syndrome is not necessarily present in its entirety in *Z. mays*.

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Die invloed van irradiansie, beligtingsduur, en verminderde water- en mineraal voedingstof-voorsiening op die karboksileringsensieme van verskillende blare van *Zea mays* L.-saailinge is bestudeer. In die eerste gevormde blare het die verhouding van PEP-karboksilase tot RuBP-karboksilase onder hoë irradiansie verhoog, en die verhouding het gedurende die fotoperiode opvallend verander. Die weerhouing van water en voedingstowwe het by al die blare van 3-week-oue plantjies die aktiwiteit van hul PEP-karboksilase meer verlaag as dié van hul RuBP-karboksilase. Die eerste fotosinteseprodukte en die $\delta^{13}C$-waarde van 3-week-oue plantjies is ook ondersoek. In al die blare is die $^{14}CO_2$ hoofsaaklik in C_4-sure ingebou, en die $\delta^{13}C$-waarde van al die blare was $-11{,}5‰$. Die resultate toon dat die groeitoestande die fotosintetiese eienskappe van die onderskeie blare beïnvloed en dat *Z. mays* nie noodwendig in sy geheel die C_4-sindroom openbaar nie.

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Introduction

Although *Zea mays* is classified as a C_4 photosynthetic plant (Black 1973), it has been shown that the first-formed leaves of plants grown under specific environmental conditions lack certain C_4 characteristics (Crespo *et al.* 1979). Initial enzymatic studies on the individual leaves of phytotron-grown *Z. mays* seedlings showed that the ratio of the carboxylating enzymes, phosphoenolpyruvate (PEP) carboxylase:ribulose-1,5-biphosphate (RuBP) carboxylase, differed in the first-formed leaves and later-developed leaves (Crespo *et al.* ibid.). The first-formed (lowermost) leaves of the seedlings consistently exhibited PEP carboxylase:RuBP carboxylase ratios <1, while the later-developed leaves always exhibited ratios >1, irrespective of leaf age. These ratios are typical of C_3 and C_4 photosynthetic plants, respectively (Black *et al.* 1973; Kestler *et al.* 1975). The CO_2 compensation point, net photosynthetic rate and ultrastructure were also shown to differ according to leaf position (Crespo *et al.* ibid.).

In this study, the effects of irradiance, illumination time and water and nutrient stress on the activity of the carboxylating enzymes of phytotron-grown *Z. mays* were studied. In addition, the $\delta^{13}C$ values and the initial $^{14}CO_2$-labelling patterns of individual leaves were determined.

Materials and Methods

Plant material

Seedlings of *Zea mays* L. (v. Kalahari Early Pearl) were grown in river-washed sand, and supplied with Long Ashton nutrient medium (Hewitt 1952) containing 200 mg dm^{-3} nitrogen (as nitrate) daily. The phytotron chamber housing the plants provided day/night temperatures of 28/21 °C and a 14-h photoperiod. The quantum flux density at plant height was 400 $\mu E\ m^{-2}s^{-1}$, except where otherwise stated.

Each leaf of the plant was assayed separately. The leaves were numbered from the base upwards, no. 1 corresponding to the lowermost leaf (first true leaf). Leaves were sampled 4 h after commencement of the photoperiod, except where the effect of illumination time was studied. The age of the plants used in each experiment is specified below.

Extraction and assay of enzymes

Ribulose-1,5-biphosphate (RuBP) carboxylase and

phosphoenolpyruvate (PEP) carboxylase were assayed in whole-leaf extracts. Freshly sampled leaf material (0,5 g) was ground in 10 cm^3 medium containing 0,15 mol dm^{-3} Tris-HCl buffer (pH 7,5), 0,01 mol EDTA dm^{-3}; 0,001 mol $MgCl_2$ dm^{-3}; 0,01 mol KCl dm^{-3} and 10 mmol dithiothreitol dm^{-3} (Fair *et al.* 1973). The homogenate was squeezed through cheese-cloth and centrifuged at 1 000 *g* for 5 min. The supernantant was used as the crude enzyme extract. All these procedures were carried out at 0–4 °C.

Irradiance study

The 2-week old plants used in this study were initially grown at 400 μE m^{-2} s^{-1} for a period of 6 days, after which the irradiance was increased to 800 μE m^{-2} s^{-1}, until the sampling date. The higher illumination was achieved in two different ways: 1) by placing the plants nearer the light bank of the phytotron chamber, which contained Sylvania VHO cool white reflectorized fluorescent tubes and 60 W tungsten strip lights, and 2) by supplying additional Osram 150 W incandescent lamps. The day/night temperatures were maintained at 28/21 °C.

Illumination time

Plants were grown at 400 μE m^{-2} s^{-1}, under a 14-h photoperiod. The individual leaves were sampled and assayed separately at 1 h, 4 h, 7 h and 10 h after commencement of the light period. Four-week old plants were used, in which the first-formed leaf (no. 1) had already senesced.

Deprivation of water and nutrients

The experimental plants were deprived of nutrient solution for 2 days prior to sampling, while the control plants received Long Ashton nutrient solution daily.

The activities of the carboxylating enzymes were determined for the individual leaves in both groups of plants. The leaves were sampled and assayed at 4 h, 7 h and 10 h after the commencement of the light period. The age of the plants used in this study was 3 weeks.

$^{13}C/^{12}C$ ratio

Leaves 1 to 5 from fifteen 3-week old plants were sampled, and pooled separately according to their positions on the stem. The leaf material was dried in an oven at 100 °C for 36 h, and then combusted at 800 °C.

Collection of the CO_2 and determination of the $\delta^{13}C$ value was done using the method of Winter *et al.* (1976). The samples were analysed using a Micromass 602C mass spectrometer.

Initial $^{14}CO_2$-fixation products

Detached leaves from positions 1 and 5 (from 3-week old plants) were allowed to photosynthesize in a closed system. Incorporation of a Uras-1 Infra-red Gas Analyzer permitted continuous monitoring of the CO_2 concentration within the system. The $^{14}CO_2$ was generated and released into circulation by the addition of lactic acid to 2 cm^3 of $NaH^{14}CO_3$ solution (0,007 mmol $NaH^{14}CO_3$ with a specific activity of 22,2 × 10^8 Bq $mmol^{-1}$). The final CO_2 concentration was 300–350 mm^3 dm^{-3}. The air in the system was allowed to equilibrate for 1 h prior to commencing the labelling with $^{14}CO_2$.

The leaves were exposed to $^{14}CO_2$ for 10 s (pulse) and immediately killed in liquid nitrogen. Other leaves were exposed to $^{14}CO_2$ for 10 s and then maintained in air at the same irradiance for 20 s (chase), after which they were immediately killed in liquid nitrogen. The irradiance during the pulse-chase was 1 700 μE m^{-2} s^{-1} supplied by a 400 W Siemens horizontal Waton mercury halide lamp.

Extraction, fractionation and identification of the labelled organic compounds namely, amino acids, sugars and organic acids (mainly 3-PGA and RuBP) were carried out according to the method used by Cresswell *et al.* (1979).

Results

Irradiance

At 800 μE m^{-2} s^{-1}, the first-formed leaves of 2-week old plants exhibited PEP carboxylase : RuBP carboxylase ratios of 1 or >1 (Figure 1 A & B). This is in contrast with the previous observation (Crespo *et al.* 1979) that the ratio in the first-formed leaves of plants grown at 400 μE m^{-2} s^{-1}, was consistently <1, while in the later-developed leaves it was consistently >1.

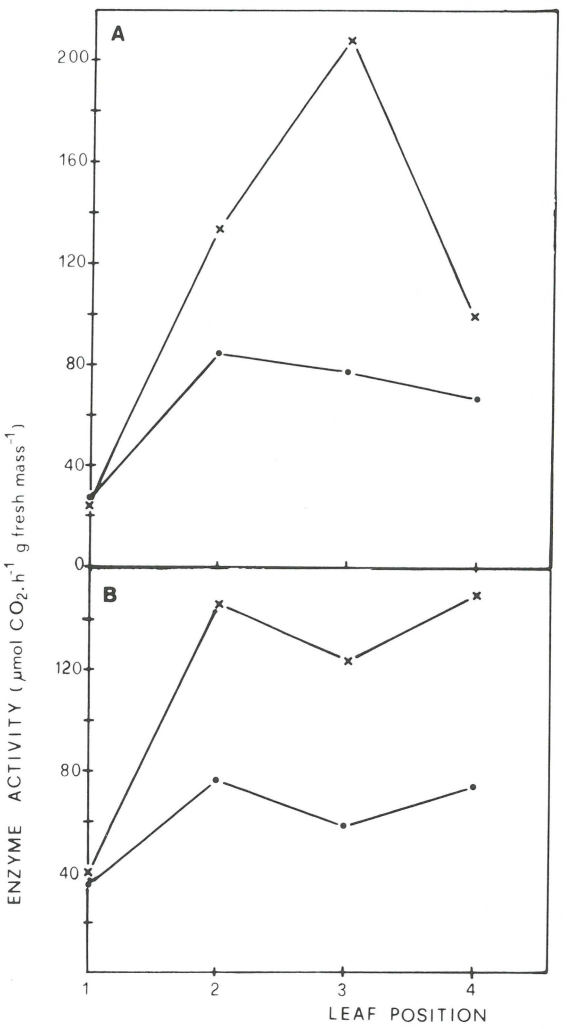

Figure 1 Ribulose biphosphate carboxylase and phosphoenolpyruvate carboxylase activities of 2-week old plants grown at 800 μE m^{-2} s^{-1}. A, additional lamps supplied; B, plants placed nearer the light bank of the growth chamber. The enzyme assays were done at 4 h after commencement of the photoperiod. (·—· RuBP carboxylase; x—x PEP carboxylase.)

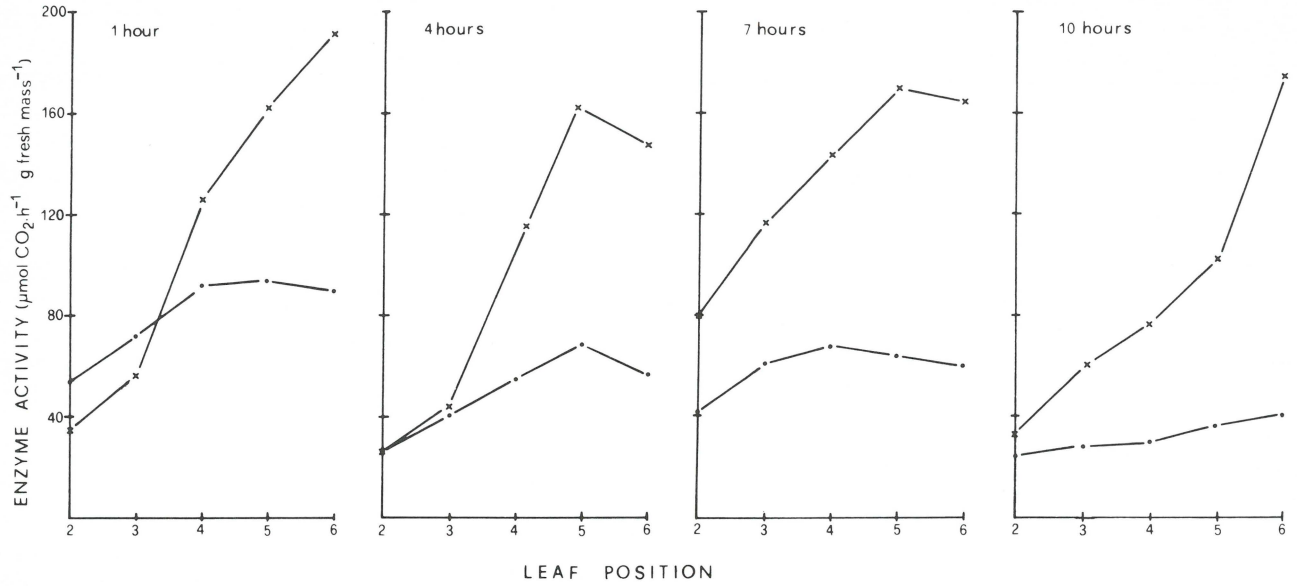

Figure 2 Influence of illumination time on the activities of ribulose biphosphate carboxylase and phosphoenolpyruvate carboxylase in 4-week old plants grown at 400 μ E m^{-2} s^{-1}. Leaves were sampled and assayed at 1, 4, 7 and 10 h after commencement of the photoperiod. (·—· RuBP carboxylase; x——x PEP carboxylase.)

Effect of illumination time

The variation in the activity of the carboxylating enzymes at 1 h, 4 h, 7 h and 10 h after commencement of the light period is illustrated in Figure 2. At 1 h the PEP carboxylase : RuBP carboxylase ratio in the lower leaves (nos. 2 and 3) was typical of that exhibited by C_3 plants, while the ratio in leaves 4 – 6 was typical of C_4 plants (Black *et al.* 1973; Kestler *et al.* 1975). At 4 h, leaf 2 showed a ratio of 1, while in leaves 3 – 6 the ratio was higher than in corresponding leaves at 1 h. It was shown previously that in younger plants (before the first-formed leaf had senesced), leaves 1 and 2 consistently, and occasionally 1 – 3, exhibited a typical C_3 ratio of PEP carboxylase : RuBP carboxylase (Crespo *et al.* 1979). At 7 h and 10 h the ratio in leaves 2 and 3 remained >1, reaching its peak at 7 h.

Water and nutrient deprivation

The activities of both carboxylating enzymes were consistently lower in the plants deprived of nutrient solution compared with the control plants (Figure 3 A & B). The PEP carboxylase activities were particularly low in the deprived plants, resulting after 7 h of illumination in a PEP carboxylase:RuBP carboxylase ratio lower than unity in leaves 2 – 4, and a reduced value for this ratio in leaf 5. This was the first instance in which a PEP carboxylase : RuBP carboxylase ratio lower than unity was recorded for leaf 4.

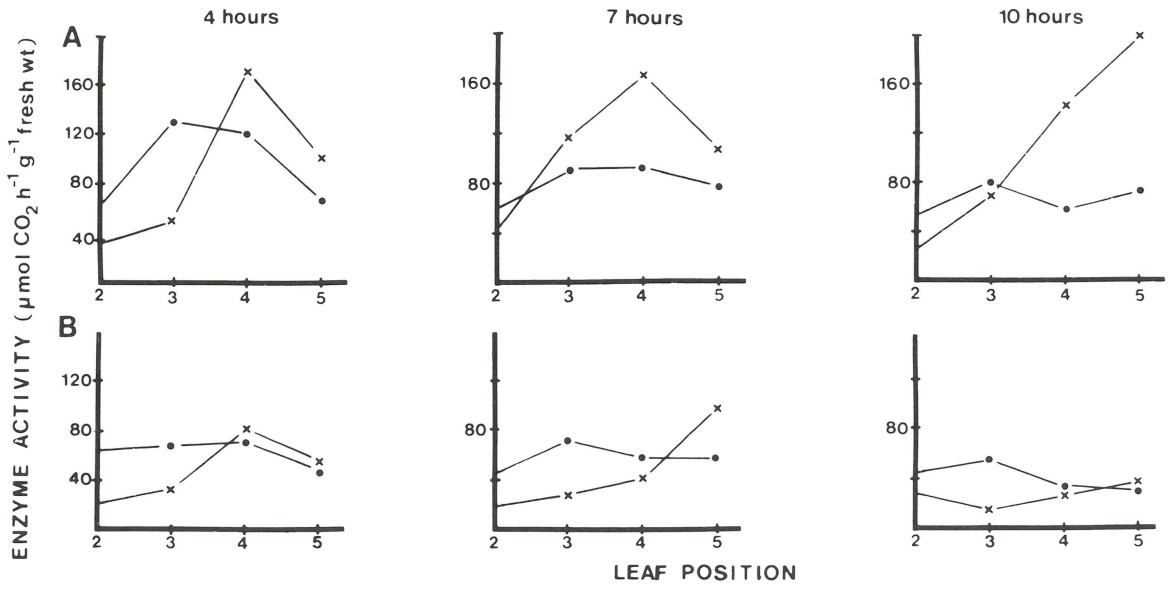

Figure 3 Carboxylating enzyme activity of 3-week old plants following a short-term deprivation of nutrient solution. The control plants (A) received nutrient solution daily, the experimental plants (B) were deprived of nutrient solution for two days prior to the assays. (·——· RuBP carboxylase; x——x PEP carboxylase.)

$^{13}C/^{12}C$ ratio

Leaves 1 to 5 all exhibited $\delta^{13}C$ values of $-11{,}5‰$, which falls within the range of values typical of C_4 plants (Smith & Epstein 1971; Ludlow *et al.* 1976). These results do not correlate with the differences in the PEP carboxylase : RuBP carboxylase ratio, CO_2 compensation point and ultrastructure previously reported for leaves of different positions in *Z. mays* seedlings (Crespo *et al.* 1979).

Initial $^{14}CO_2$-fixation products

During short-term exposure to $^{14}CO_2$ (10 s), both leaf 1 and leaf 5 incorporated the radioactivity into C_4 acids, malate being the predominant acid labelled (Table 1). The labelling pattern during the chase was typical of that found in C_4 plants (Raghavendra & Das 1978; Black *et al.* 1973), but transfer of radioactivity to Calvin cycle intermediates was slower in leaf 1.

Table 1 Photosynthetic ^{14}C-labelling pattern of leaves 1 and 5 after a 10-s pulse and the subsequent 20-s chase

	Radioactivity incorporated in counts min^{-1} (g fresh mass)$^{-1} \times 10^4$			
Leaf No.	Malate	Aspartate	3-PGA and sugar-phosphates	Sugars
After 10-s exposure to $^{14}CO_2$				
1	57	2	0,2	0,8
5	47	10	0,2	0,6
After 20-s chase				
1	26	2	0,2	16
5	8	5	0,6	30

Discussion and Conclusions

Effects of irradiance and illumination time on the activity of the carboxylating enzymes

The results indicate that irradiance and illumination time affect the activity of PEP carboxylase to a greater extent than that of RuBP carboxylase, in the first-formed leaves of *Z. mays*. This is in accordance with the report of Hatch *et al.* (1969), that the PEP carboxylase activity in maize and *Amaranthus palmeri* grown under high irradiance was higher than in plants grown under low light conditions. These authors observed that PEP carboxylase activity increased 5- to 10-fold in plants that were transferred from low to high irradiance whereas RuBP carboxylase activity remained unchanged. The increased activity of PEP carboxylase in maize receiving high illumination during growth, is largely the result of increased *de novo* synthesis of the enzyme protein (Hague & Sims 1980).

The variation in PEP carboxylase activity exhibited by the youngest leaves (Figures 1 – 3) has been previously explained in terms of their relative state of immaturity (Crespo *et al.* 1979).

Effects of water and nutrient deprivation on the activity of the carboxylating enzymes

Deprivation of nutrient solution depressed the activity of both carboxylating enzymes, but the activity of PEP carboxylase was affected to a greater extent than that of RuBP carboxylase. This resulted in a general reduction of the PEP carboxylase : RuBP carboxylase ratio, and even leaf 4 exhibited a ratio <1. A ratio lower than unity for leaf 4 has not been previously recorded (Crespo *et al.* 1979).

The above recorded effects of limiting the nutrient and water supply are in accordance with reports by other workers, that:

(i) mineral nutrition affects PEP carboxylase activity more markedly than RuBP carboxylase activity. Tew (1976) showed that increased nitrogen concentration, in the form of ammonia, resulted in a greater increase in PEP carboxylase than RuBP carboxylase activity in *Z. mays, Eragrostis curvula* and *Hyparrhenia hirta*; Shomer-Ilan & Waisel (1973) reported that in *Z. mays* and *Aleuropus litoralis*, increased concentration of NaCl in the nutrient medium resulted in increased activity of PEP carboxylase but not of RuBP carboxylase.

(ii) the mesophyll cells are more prone to damage under conditions of water stress than the bundle sheath cells (Alberte *et al.* 1977; Giles *et al.* 1974; Mittleheuser 1977). The latter supports the results of the present study, since the enzyme PEP carboxylase has been shown to be present in the mesophyll cells (Black *et al.* 1971; Gutierrez *et al.* 1974).

$^{13}C/^{12}C$ ratio and initial $^{14}CO_2$-fixation products

The values obtained for the $^{13}C/^{12}C$ ratio of leaves 1 to 5, and the $^{14}CO_2$-labelling pattern of leaves 1 and 5, were typical of C_4 plants. These results are surprising in view of the previous findings that leaves 1 – 3 exhibit PEP carboxylase : RuBP carboxylase ratios typical of C_3 plants, and that leaf 1 had a CO_2 compensation point of 43 mm^3 dm^{-3} (Crespo *et al.* 1979).

It has been shown that the low PEP carboxylase activities recorded for the first-formed leaves are not due to endogenous inhibitors released during the *in vitro* assay, since partial purification of the enzyme with Sephadex G-25 and 2% polyvinylpyrrolidone did not alter the ratio of the carboxylating enzymes obtained in the crude extracts (Crespo 1979).

The results obtained in the studies on illumination and water and nutrient stress, indicate that the enzyme PEP carboxylase is more sensitive to changed environmental conditions, than RuBP carboxylase.

The results presented here, together with those previously reported (Crespo *et al.* 1979), suggest that the C_4 syndrome is a variable system, certain characteristics of which appear to be determined by leaf position and environmental conditions. It is concluded that the presence of certain C_4 characteristics does not imply the presence of the entire C_4 syndrome.

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